

FORM PTO-1390 (REV 10-94)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 9548.50USWO
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) unknown 09/787328
INTERNATIONAL APPLICATION NO. PCT/CN99/00139	INTERNATIONAL FILING DATE September 6, 1999	PRIORITY DATE CLAIMED September 22, 1998	
TITLE OF INVENTION NEW HUMAN HEPATOMA-DERIVED GROWTH FACTOR ENCODING SEQUENCE AND POLYPEPTIDE ENCODED BY SUCH DNA SEQUENCE AND PRODUCING METHOD THEREOF			
APPLICANT(S) FOR DO/EO/US YU et al.			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11. to 16. below concern document(s) or information included:			
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: PCT/IPEA/409; English translation of PCT/IPEA/409; PCT/ISA/210 			

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5) unknown 09/787328		INTERNATIONAL APPLICATION NO PCT/CN99/00139		ATTORNEY'S DOCKET NUMBER 9548.50USWO	
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17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)): Search Report has been prepared by the EPO or JPO.....\$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)).....\$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO\$1000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e))					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	14 -20 =	0	X \$18.00		
Independent claims	3 -3 =	0	X \$80.00		
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$260.00	
TOTAL OF ABOVE CALCULATIONS =				\$1000.00	
Reduction by 1/2 for filing by small entity, if applicable. Small entity status is claimed pursuant to 37 CFR 1.27				\$500.00	
SUBTOTAL =				\$500.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+ \$	
TOTAL NATIONAL FEE =				\$500.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+ \$40.00	
TOTAL FEES ENCLOSED =				\$540.00	
				Amount to be:	
				refunded	\$
				charged	\$


a. ☒ Check(s) in the amount of \$540.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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REGISTRATION NUMBER: 30,422

**NEW HUMAN HEPATOMA-DERIVED GROWTH FACTOR ENCODING SEQUENCE
AND POLYPEPTIDE ENCODED BY SUCH DNA SEQUENCE
AND PRODUCING METHOD THEREOF**

5 Field of invention

This invention relates to the field of genetic engineering, and, in particular, relates to the nucleotide sequence of a novel human gene. More particularly, this invention relates to the cDNA sequence of a novel type II human Hepatoma-derived Growth Factor (HDGF2), which is a homologue of type I HDGF. The invention also relates to the polypeptides encoded by the nucleotide sequence, the uses of these polynucleotides and polypeptides, and the methods for producing them.

Prior art

It is revealed that the regulation of cell growth is mediated by a series of cascade reactions triggered by the interaction between a variety of cytokines and their specific receptors on membrane surfaces. In tumor cells, some steps of cascade reactions appear to be out of control, which results in continuous cellular proliferation. In hepatoma cells, several autocrine and paracrine cell factors were found (Proc. Natl. Acad. Sci. 83:2448-2452, 1986; Proc. Natl. Acad. Sci. 86:7432-7436, 1989; Cell 61: 1137-1146, 1990). Hepatoma-derived Growth Factor (HDGF) was a cytokine identified from human hepatoma-derived cell line HuH-7 cultured in serum-free medium. HDGF had the heparin-binding activity and stimulated the DNA synthesis in Swiss 3T3 cell (J. Biol. chem. 269 (40): 25143-25149, 1994).

In 1989, HDGF was first partially purified from HuH-7 cells and characterized by Nakamura et.al. (Clin. Chim. Acta. 183:273-284, 1989). This research group cloned the full length HDGF cDNA sequence in 1994 (J. Biol. Chem. 269(40): 25143-25149, 1994). In 1997, this group found the mouse homologue of human HDGF as well as other two members of the gene family, HRP-1 and HRP-2. They all had a highly conserved N-terminal of 98 amino acids. (Biochem. Biophys. Res. Commun. 238: 26-32, 1997).

Prior to this invention, none has disclosed human HDGF2 of the present application concerns, which is another member of the human HDGF family.

Summary of Invention

One purpose of the invention is to provide a new polynucleotide which encodes a homologue of HDGF. In the invention, the gene of said homologue of HDGF is named HDGF2.

Another purpose of the invention is to provide a novel protein, which is named HDGF2.

Still another purpose of the invention is to provide a new method for preparing said new HDGF2 protein by recombinant techniques.

The invention also relates to the uses of said HDGF2 protein and its coding sequence.

In one aspect, the invention provides an isolated DNA molecule, which comprises a nucleotide sequence encoding a polypeptide having human HDGF2 protein activity, wherein said nucleotide sequence shares at least 70% homology to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3, or said

nucleotide sequence can hybridize to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3 under moderate stringency. Preferably, said nucleotide sequence encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 4. More preferably, the sequence comprises the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3.

5 Further, the invention provides an isolated HDGF2 polypeptide, which comprises a polypeptide having the amino acid sequence of SEQ ID NO: 4, its active fragments, and its active derivatives. Preferably, the polypeptide is a polypeptide having the amino acid sequence of SEQ ID NO: 4.

The invention also provides a vector comprising said isolated DNA.

The invention further provides a host cell transformed with said vector.

10 In another aspect, the invention provides a method for producing a polypeptide with the activity of HDGF2 protein, which comprises:

(a) forming a HDGF2 protein expression vector comprising the nucleotide sequence encoding the polypeptide having the activity of HDGF2 protein, wherein said nucleotide sequence is operably linked with an expression regulatory sequences, and said nucleotide sequence shares at least 70% homology to the nucleotide sequence of positions 121-732 in SEQ ID NO: 3;

15 (b) introducing the vector of step (a) into a host cell, thereby forming a recombinant cell of HDGF2 protein;

(c) culturing the recombinant cell of step (b) under the conditions suitable for the expression of HDGF2 polypeptides;

20 (d) isolating the polypeptides having the activity of HDGF2 protein.

In one embodiment of the present invention, the isolated polynucleotide has a full length of 1024 nucleotides, whose detailed sequence is shown in SEQ ID NO: 3. The open reading frame (ORF) is located at nucleotides 121-732.

25 In the present invention, the term "isolated" or "purified" or "substantially pure" DNA refers to a DNA or fragment which has been isolated from the sequences which flank it in a naturally occurring state. The term also applies to DNA or DNA fragment which has been isolated from other components naturally accompanying the nucleic acid and from proteins naturally accompanying it in the cell.

In the present invention, the term "HDGF2 protein encoding sequence" or "HDGF2 polypeptide encoding sequence" refers to a nucleotide sequence encoding a polypeptide having the activity of HDGF2 protein, such as the nucleotide sequence of positions 121-732 in SEQ ID NO: 3 or its degenerate sequence. The degenerate sequences means the sequences formed by replacing one or more codons in the ORF of 121-732 in SEQ ID NO: 3 with degenerate codes which encode the same amino acid. Because of the degeneracy of codon, the sequence having a homology as low as about 70% to the sequence of nucleotides 121-732 in SEQ ID NO: 3 can also encode the sequence shown in SEQ ID NO: 4. The term also refers to the nucleotide sequences that hybridize to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3 under moderate stringency or preferably under high stringency. In addition, the term also refers to the sequences having a homology of at least 70%, preferably 80%, more preferably 90% to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3.

The term also refers to variants of the sequence in SEQ ID NO: 3, which are capable of encoding a protein having the same function as human HDGF2 protein. These variants includes, but are not limited to, deletions, insertions and/or substitutions of several nucleotides (typically 1-90, preferably 1-60, more preferably 1-20, and most preferably 1-10) and additions of several nucleotides (typically less than 60, preferably 30, more preferably 10, most preferably 5) at 5' end and/or 3' end.

In the present invention, "substantially pure" proteins or polypeptides refers to those which occupy at least 20%, preferably at least 50%, more preferably at least 80%, most preferably at least 90% of the total sample material (by wet weight or dry weight). Purity can be measured by any appropriate method, e.g., in the case of polypeptides by column chromatography, PAGE or HPLC analysis. A substantially purified polypeptides is essentially free of naturally associated components.

In the present invention, the term "HDGF2 polypeptide" or "HDGF2 protein" refers to a polypeptide having the activity of HDGF2 protein comprising the amino acid sequence of SEQ ID NO: 4. The term also comprises the variants of said amino acid sequence which have the same function of human HDGF2. These variants include, but are not limited to, deletions, insertions and/or substitutions of several amino acids (typically 1-50, preferably 1-30, more preferably 1-20, most preferably 1-10), and addition of one or more amino acids (typically less than 20, preferably less than 10, more preferably less than 5) at C-terminal and/or N-terminal. For example, the protein functions are usually unchanged when an amino residue is substituted by a similar or analogous one. Further, the addition of one or several amino acids at C-terminal and/or N-terminal will not change the function of protein. The term also includes the active fragments and derivatives of HDGF2 protein.

The variants of polypeptide include homologous sequences, allelic variants, natural mutants, induced mutants, proteins encoded by DNA which hybridizes to HDGF2 DNA under high or low stringency conditions as well as the polypeptides or proteins retrieved by antisera raised against HDGF2 polypeptide. The present invention also provides other polypeptides, e.g., fusion proteins, which include the HDGF2 polypeptide or fragments thereof. In addition to substantially full-length polypeptide, the soluble fragments of HDGF2 polypeptide are also included. Generally, these fragments comprise at least 10, typically at least 30, preferably at least 50, more preferably at least 80, most preferably at least 100 consecutive amino acids of HDGF2 polypeptide.

The present invention also provides the analogues of HDGF2 protein or polypeptide. Analogues can differ from naturally occurring HDGF2 polypeptide by amino acid sequence differences or by modifications which do not affect the sequence, or by both. These polypeptides include genetic variants, both natural and induced. Induced variants can be made by various techniques, e.g., by random mutagenesis using irradiation or exposure to mutagens, or by site-directed mutagenesis or other known molecular biologic techniques. Also included are analogues which include residues other than those naturally occurring L-amino acids (e.g., D-amino acids) or non-naturally occurring or synthetic amino acids (e.g., beta- or gamma-amino acids). It is understood that the polypeptides of the invention are not limited to the representative polypeptides listed hereinabove.

Modifications (which do not normally alter primary sequence) include in vivo, or in vitro chemical derivation of polypeptides, e.g., acetylation, or carboxylation. Also included are modifications of

glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in the further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation (e.g., mammalian glycosylating or deglycosylating enzymes). Also included are sequences which have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, phosphothreonine, as well as sequences which have been modified to improve their resistance to proteolytic degradation or to optimize solubility properties.

The invention also includes antisense sequence of the sequence encoding HDGF2 polypeptide. Said antisense sequence can be used to inhibit expression of HDGF2 in cells.

The invention also includes probes, typically having 8-100, preferably 15-50 consecutive nucleotides. These probes can be used to detect the presence of nucleic acid molecules coding for HDGF2 in samples.

The present invention also includes methods for detecting HDGF2 nucleotide sequences, which comprises hybridizing said probes to samples, and detecting the binding of the probes. Preferably, the samples are products of PCR amplification. The primers in PCR amplification correspond to coding sequence of HDGF2 polypeptide and are located at both ends or in the middle of the coding sequence. In general, the length of the primers is 20 to 50 nucleotides.

A variety of vectors known in the art, such as those commercially available, are useful in the invention.

In the invention, the term "host cells" includes prokaryotic and eukaryotic cells. The common prokaryotic host cells include Escherichia coli, Bacillus subtilis, and so on. The common eukaryotic host cells include yeast cells, insect cells, and mammalian cells. Preferably, the host cells are eukaryotic cells, e.g., CHO cells, COS cells, and the like.

In another aspect, the invention also includes antibodies, preferably monoclonal antibodies, which are specific for polypeptides encoded by HDGF2 DNA or fragments thereof. By "specificity", it is meant an antibody which binds to the HDGF2 gene products or a fragments thereof. Preferably, the antibody binds to the HDGF2 gene products or a fragments thereof and does not substantially recognize nor bind to other antigenically unrelated molecules. Antibodies which bind to HDGF2 and block HDGF2 protein and those which do not affect the HDGF2 function are included in the invention. The invention also includes antibodies which bind to the HDGF2 gene product in its unmodified as well as modified form.

The present invention includes not only intact monoclonal or polyclonal antibodies, but also immunologically-active antibody fragments, e.g., a Fab' or (Fab)₂ fragment, an antibody light chain, an antibody heavy chain, a genetically engineered single chain Fv molecule (Lander, et al., US Pat No. 4,946,778), or a chimeric antibody, e.g., an antibody which contains the binding specificity of a murine antibody, but the remaining portion of which is of human origin.

The antibodies in the present invention can be prepared by various techniques known to those skilled in the art. For example, purified HDGF2 gene products, or its antigenic fragments can be administered to animals to induce the production of polyclonal antibodies. Similarly, cells expressing HDGF2 or its antigenic fragments can be used to immunize animals to produce antibodies. Antibodies of the invention can be monoclonal antibodies which can be prepared by using hybridoma technique (See Kohler, et al., Nature, 256; 495, 1975; Kohler, et al., Eur. J. Immunol. 6: 511, 1976; Kohler, et al., Eur. J. Immunol. 6: 292, 1976; Hammerling, et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, N.Y., 1981).

Antibodies of the invention comprise those which block HDGF2 function and those which do not affect HDGF2 function. Antibodies in the invention can be produced by routine immunology techniques and using fragments or functional regions of HDGF2 gene product. These fragments and functional regions can be prepared by recombinant methods or synthesized by a polypeptide synthesizer. Antibodies binding to unmodified HDGF2 gene product can be produced by immunizing animals with gene products produced by prokaryotic cells (e.g., *E. coli*); antibodies binding to post-translationally modified forms thereof can be acquired by immunizing animals with gene products produced by eukaryotic cells (e.g., yeast or insect cells).

The full length human HDGF2 nucleotide sequence or its fragment of the invention can be prepared by PCR amplification, recombinant method and synthetic method. For PCR amplification, one can obtain said sequences by designing primers based on the nucleotide sequence disclosed in the invention, especially the sequence of ORF, and using cDNA library commercially available or prepared by routine techniques known in the art as a template. When the sequence is long, it is usually necessary to perform two or more PCR amplifications and link the amplified fragments together in the correct order.

Once the sequence is obtained, a great amount of the sequences can be produced by recombinant methods. Usually, said sequence is cloned in a vector which is then transformed into a host cell. Then the sequence is isolated from the amplified host cells using conventional techniques.

Further, the sequence can be produced by synthesis. Typically, several small fragments are synthesized and linked together to obtain a long sequence. At present, it is completely feasible to chemically synthesize the DNA sequence encoding the protein of the invention, or the fragments or derivatives thereof. In addition, the mutation can be introduced into the sequence of the protein by chemical synthesis.

In addition to recombinant techniques, the protein fragments of the invention may also be prepared by direct chemical synthesis using solid phase synthesis techniques (Stewart et al., (1969) *Solid-Phase Peptide Synthesis*, WH Freeman Co., San Francisco; Merrifield J. (1963), *J. Am. Chem. Assoc.* 85: 2149-2154). In vitro protein synthesis can be performed manually or automatically, e.g., using a Model 431 Peptide Synthesizer (Applied Biosystems, Foster City, CA). The fragments of protein of the invention can be synthesized separately and linked together using chemical methods so as to produce full-length molecule.

The sequences encoding the protein of the present invention are also valuable for gene mapping. For example, the accurate chromosome mapping can be performed by hybridizing cDNA clones to a chromosome in metaphase. This technique can use cDNA as short as about 500bp, or as long as about 2000bp, or more. For details, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, e.g., *Mendelian Inheritance in Man* (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis.

Then, the differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any

normal individual, then the mutation is likely to be the causative agent of the disease.

The substances which act with the HDGF2, e.g., receptors, inhibitors and antagonists, can be screened out by various conventional techniques, using the protein of the invention.

The protein, antibody, inhibitor, antagonist or receptor of the invention provide different effects when administrated in therapy. Usually, these substances are formulated with a non-toxic, inert and pharmaceutically acceptable aqueous carrier. The pH typically ranges from 5 to 8, preferably from about 6 to 8, although pH may alter according to the property of the formulated substances and the diseases to be treated. The formulated pharmaceutical composition is administrated in conventional routine including, but not limited to, intramuscular, intraperitoneal, subcutaneous, intracutaneous, or topical administration.

As an example, the human HDGF2 protein of the invention may be administrated together with the suitable and pharmaceutically acceptable carrier. The examples of carriers include, but are not limited to, saline, buffer solution, glucose, water, glycerin, ethanol, or the combination thereof. The pharmaceutical formulation should be suitable for the delivery method. The human HDGF2 protein of the invention may be in the form of injections which are made by conventional methods, using physiological saline or other aqueous solution containing glucose or auxiliary substances. The pharmaceutical compositions in the form of tablet or capsule may be prepared by routine methods. The pharmaceutical compositions, e.g., injections, solutions, tablets, and capsules, should be manufactured under sterile conditions. The active ingredient is administrated in therapeutically effective amount, e.g., from about 1 μ g to 5mg per kg body weight per day. Moreover, the polypeptide of the invention can be administrated together with other therapeutic agent.

When the human HDGF2 polypeptides of the invention are used as a pharmaceutical, the therapeutically effective amount of the polypeptides are administrated to mammals. Typically, the therapeutically effective amount is at least about 10 μ g/kg body weight and less than about 8 mg/kg body weight in most cases, and preferably about 10 μ g-1mg/kg body weight. Of course, the precise amount will depend upon various factors, such as delivery methods, the subject health, and the like, and is within the judgment of the skilled clinician.

Description of Drawings

Fig. 1 shows an alignment comparison of nucleotide sequences of HDGF2 of the invention and mouse HDGF2. The identical nucleotides are indicated by "|".

Fig. 2 shows an alignment comparison of amino acid sequences of HDGF2 of the invention and mouse HDGF2. The identical and similar amino acids are indicated by "|" and ".", respectively.

In one embodiment, the cDNA sequence of HDGF2 was obtained as follows: human testis λ gt 11 cDNA library (Clontech) was used as a template and PCR was carried out with the synthetic forward primer A1 :5'-ACCGCTCGTC CGCCCGGCTT GAG-3' and reverse primer B :5'-GATCCTAGAC ATGTATAAGT CTGCGC-3'. Target fragments of 1024bp were obtained. The sequencing of the PCR product gave the full length cDNA sequence shown in SEQ ID NO: 3.

Hepatoma-derived Growth Factor (HDGF) is a heparin-binding protein isolated from human hepatoma-derived cell line HuH-7. HDGF has the activity of stimulating cell growth and promoting the growth of

fibroblast and some heptoma cells (J.Biol.Chem. 269(40): 25143-25149, 1994). It is expressed in human heart, brain, lung, liver, etc., and several tumor-derived cell lines (J.Biol.Chem. 269(40): 25143-25149, 1994). The expression patterns of the HDGF gene family members are different. However, they are all enriched in testis and the 5'-untranslated region contains GC-rich nucleotide sequences (GC content >70%) (Biochem. Biophys. Res. Commun. 238: 26-32, 1997), suggesting their potential important roles in male germ-cell development. They may also relate to DNA methylation, chromatin conformation, and translational regulation (J. Cell. Biol. 115: 887-903, 1990; Cell 62: 503-514, 1990). Although HDGF protein is located mainly in cytoplasm (J. Biol. Chem. 269(40): 25143-25149, 1994), the amino acid sequences of family members all contain a putative Nuclear Localization Signal (NLS), and none have any signal peptide sequence, which suggests they may play a role in nucleus. Furthermore, the acidic amino acid sequence in the C-terminus of HDGF shares a high homology to that of HMG-1/-2 of HMG family (This sequence is known to be a histone-binding region in HMG-1/-2) (Biochemistry 29: 4419-4423, 1990). It is likely that HDGF functions as a transcriptional factor to stimulate cell growth after internalization (Biochem. Biophys. Res. Commun. 238: 26-32, 1997). The mitogenic activity of HDGF implies the great application value of HDGF in treating pernicious oxyhepatitis and liver injury (Clin. Chim. Acta. 183L 273-284, 1989). Researches indicate that many fibroblast growth factors can be widely applied to the vascularization defects, i.e., ischemia and atherosclerosis, and to neuron development (Blood 91(10): 3527-3561, 1998; Ann. N. Y. Acad. Sci. 545: 240-252, 1998).

The invention is further illustrated by the following examples. It is appreciated that these examples are only intended to illustrate the invention, but not to limit the scope of the invention. For the experimental methods in the following examples, they are performed under routine conditions, e.g., those described by Sambrook. et al., in Molecule Clone: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press, 1989, or as instructed by the manufacturers, unless otherwise specified.

Example 1

The cloning and sequencing of HDGF2 cDNA sequence

1. Amplification with primers

The template was human testis λ gt 11 cDNA library (commercially available from Clontech). PCR was carried out with the forward primer A1: 5'-ACCGCTCGTC CGCCCGGCTT GAG-3' (SEQ ID NO: 1) and reverse primer A2: 5'-GATCCTAGAC ATGTATAAGT CTGCGC-3' (SEQ ID NO: 2). The PCR condition was 4 mins at 93°C; followed by 35 cycles with 1 min at 93°C, 1 min at 68.5°C, and 1 min at 72°C; and, finally 5 mins at 72°C. The PCR fragments were detected by electrophoresis. The target fragment was 1024bp.

2. Sequencing PCR products

The above obtained PCR products were linked with pGEM-TTM vector (Promega) and transformed into E. coli JM103. The plasmids were extracted using QIAprep Plasmid Kit (QIAGEN). The oriented serial deletion of the inserted fragments was carried out with Double-Stranded Nested Deletion Kit

(Pharmacia), and the deletants were quickly identified by PCR and arranged in order. The deletants successively cut-off were sequenced with SequiTherm EXCEL™ DNA Sequencing Kit (Epicentre Technologies). A full length cDNA sequence of 1024bp was obtained by overlapping the sequences with computer software. The detailed sequence is shown in SEQ ID NO: 3 with an open reading frame (ORF) located at nucleotides 121-732.

According to the resultant full-length cDNA sequence, the amino acid sequence of HDGF2 was deduced, having 203 amino acid residues totally. See SEQ ID NO: 4 for its amino acid sequence in details.

Example 2

Homologous comparison

The full length cDNA sequence of human HDGF2 and the encoded protein were used for homologous searching Non-redundant GenBank + EMBL + DDBJ + PDB and on-redundant GenBank CDS translations + PDB + SwissProt + Spupdate + PIR databases by BLAST algorithm. The result showed that they shared high homology to mouse HDGF2 (dbj|D63707|MUSHDGF) gene and its encoded protein. Using PCGENE software, it was found that they shared 68.7% identity at the nucleic acid level and 53.7% identity and 9.4% similarity at the protein level (FIG.1 and FIG.2). In particular, the conserved 98-amino acid N-terminal showed 90% homology to mouse HDGF. In addition, human HDGF2 was homologous to another mouse HDGF gene (dbj|D63850|MUSHDGF) and another human HDGF gene (dbj|D16431|HUMHDGF). These abovementioned genes are regarded as a family. So the functions of the HDGF2 can be deduced from the known functions of these genes and proteins.

Hepatoma-derived Growth Factor (HDGF) is a heparin-binding protein isolated from human hepatoma-derived cell line HuH-7. HDGF has the activity of stimulating cell growth and promoting the growth of fibroblast and some heptoma cells (J.Biol.Chem. 269(40): 25143-25149, 1994). Though HDGF was firstly identified in hepatoma cells, Northern blotting analysis showed that it was expressed in human heart, brain, lung, liver, etc., and several tumor-derived cell lines (J.Biol.Chem. 269(40): 25143-25149, 1994). It needed further investigation to determine whether HDGF was expressed differently in normal cells and tumor cells (J.Biol.Chem. 269(40): 25143-25149, 1994). The functions of HDGF in hepatoma cells and its influence on hepatoma treatment would be revealed constantly as the researches were carried out. The expression patterns of the HDGF gene family members were different. However, they were all enriched in testis and the 5'-untranslated region contained GC-rich nucleotide sequences (GC content >70%) (Biochem. Biophys. Res. Commun. 238: 26-32, 1997). This property was similar to genes specifically expressed in testis or embryonic development, suggesting the potential important roles in male germ-cell development. They might also relate to DNA methylation, chromatin conformation, and translational regulation (J. Cell. Biol. 115: 887-903, 1990; Cell 62: 503-514, 1990).

It was revealed by immunofluorescence test that HDGF protein was located mainly in cytoplasm (J. Biol. Chem. 269(40): 25143-25149, 1994). The amino acid sequences of family members all contained a putative Nuclear Localization Signal (NLS), and none had any signal peptide sequence, which suggested they may play a role as a nucleoprotein. Fibroblast Growth Factor (FGF) was located in nuclear by this signal sequence to exert its mitogenic activity. Furthermore, the acidic amino acid sequence in the C-

terminus of HDGF shared a high homology to that of HMG-1/-2 of HMG family and said sequence was known to be a histone-binding region in HMG-1/-2. (Biochemistry 29: 4419-4423, 1990). Summing up, it was likely that HDGF functions as a transcriptional factor to stimulate cell growth after internalization (Biochem. Biophys. Res. Commun. 238: 26-32, 1997). HDGF2 of the invention had similar activity.

5 The mitogenic activity of HDGF implied the great application value of HDGF in treating pernicious oxyhepatitis and liver injury (Clin. Chim. Acta. 183L 273-284, 1989). Researches indicated that many fibroblast growth factors were capable of promoting the growth of epithelial cells and could be widely applied to the vascularization defects, i.e., ischemia and atherosclerosis, and to neuron development (Blood 91(10): 3527-3561, 1998; Ann. N. Y. Acad. Sci. 545: 240-252, 1998). The application of HDGF1 and
10 HDGF2 of the invention in promoting the growth of fibroblast needs further study.

The HDGF2 of the invention can be used not only as a member of the family in the study of function, but also to produce fusion proteins with other proteins, such as immunoglobulins. Besides, HDGF2 can be fused with or exchange fragments with other members of the family to form new proteins. For example, the N terminal of HDGF2 can exchange with the N terminal of HDGF1 or mice HDGF to produce proteins
15 which are more active or have new properties.

The antibodies against HDGF2 can be used to screen other members of the family or to purify the related proteins such as other members of the family through affinity purification.

Example 3

20 Expression of HDGF2 in E. coli

In this example, the cDNA sequence encoding HDGF2 was amplified with oligonucleotide PCR primers corresponding to 5'- and 3'-end of said DNA sequence. The resultant HDGF2 cDNA was used as an insertion fragment.

The sequence of 5'-end oligonucleotide primer was:

25 5'-CCACGGATCCATGGCGCGTCCGCGGCCCC-3' (SEQ ID NO: 5).

This primer contained a cleavage site of restriction endonuclease BamH I, followed by 19 nucleotides of HDGF2 coding sequence starting from the start codon.

The sequence of 3'-end primer was:

30 5'-ATCCGTCGACTTAGGTCCCTTCACTGGTT-3' (SEQ ID NO: 6).

This primer contained a cleavage site of restriction endonuclease SalI, a translation terminator and partial HDGF2 coding sequence.

These cleavage sites of restriction endonuclease in primers corresponded to the cleavage sites in bacterial expression vector pQE-9 (Qiagen Inc., Chatsworth, CA). Vector pQE-9 encodes an antibiotic resistance (Amp^r), a bacterial replication origin (ori), an IPTG-adjustable promotor/operon (P/O), a
35 ribosome-binding site (RBS), a six-histidine tag (6-His) and cloning sites of restriction endonuclease.

Vector pQE-9 and insertion fragments were digested by BamHI and SalI, and then linked together, ensuring that the open reading frame started from the bacterial RBS. Then, the linkage mixture was used to transform E.coli M15/rep4 (Qiagen) containing multi-copy of plasmid pREP4 which expressed repressor of lacI and was resistant to kanamycin (Kan^r). Transformants were screened out in LB medium containing

Amp and Kan. The plasmids were extracted. The size and direction of the inserted fragments were verified by PstI digestion. The sequencing confirmed that HDGF2 cDNA fragment was correctly inserted into the vector.

The positive clones of transformant were cultured overnight in LB liquid medium supplemented with Amp (100ug/ml) and Kan (25ug/ml). The overnight culture was 1:100-1:250 diluted, inoculated into large volume medium, and cultured until the 600nm optical density (OD₆₀₀) reached 0.4-0.6. IPTG (isopropylthio-beta-D-galactoside) was added to final concentration of 1mM. By deactivating repressor of LacI, IPTG induced and promoted P/O, thereby increasing the expression of gene. The cells were cultured for another 3-4 hours, and then centrifuged (6000 X g, 20 mins). The cultures were sonicated, and cell lysate was collected and diluted with 6M guanidine hydrochloride. After clarification, the dissolved HDGF2 in solution were purified by nickel-chelated column chromatography under the conditions suitable for the tight binding of 6-His tagged protein and column. HDGF2 was eluted with 6M guanidine hydrochloride (pH 5.0). The denaturalized proteins in guanidine hydrochloride were precipitated by several methods. First, guanidine hydrochloride was separated by dialysis. Alternatively, the purified protein, which was isolated from nickel-chelated column, bound to the second column with decreased linear gradient of guanidine hydrochloride. The proteins were denatured when binding to the column. Then, the proteins were eluted with guanidine hydrochloride (pH 5.0). Finally, the soluble proteins were dialyzed with PBS, then preserved in glycerol stock solution with the final glycerol concentration of 10% (w/v).

The molecular weight of the expressed protein was about 23 kDa, as identified by 12% SDS-PAGE.

Moreover, the sequencing results of the 10 amino acids at the N- and C-terminal of the expressed protein indicated that they were identical to those in SEQ ID NO: 4.

Example 4

Expression of HDGF2 in eukaryotic cells (CHO cell line)

In this example, the cDNA sequence encoding HDGF2 was amplified with oligonucleotide PCR primers corresponding to 5'- and 3'-end of said DNA sequence. The resultant product was used as an insertion fragment.

The sequence of 5'-end oligonucleotide primer was:

5'- CCCTAAGCTTATGGCGCGTCCGCGGCCCC-3'(SEQ ID NO: 7),

This primer contained a cleavage site of restriction endonuclease HindIII, followed by 19 nucleotides of HDGF2 coding sequence starting from the start codon.

The sequence of 3'-end primer was:

5'-TTTCGGATCCTTAGGTCCTTCACTGGTT-3' (SEQ ID NO: 8)

This primer contained a cleavage site of restriction endonuclease BamHI, a translation stop codon, and partial HDGF2 coding sequence.

These cleavage sites of restriction endonuclease in primers corresponded to the cleavage sites in expression vector pcDNA3 for CHO cell. This vector encoded two kinds of antibiotic resistance (Amp^r and Neo^r), a phage replication origin (fl ori), a virus replication origin (SV40 ori), a T7 promoter, a virus promoter (P-CMV), a Sp6 promoter, a polyadenylation signal of SV40 and the corresponding polyA

sequence thereof, a polyadenylation signal of BGH and the corresponding poly A sequence thereof.

The vector pcDNA3 and insertion fragment were digested with HindIII and BamHI, and linked together. Subsequently, E.coli strand DH5 α was transformed with linkage mixture. Transformants were screened out in LB medium containing Amp. The clones containing the needed constructs were cultured overnight in LB liquid medium supplemented with Amp (100 ug/ml). Plasmids were extracted. The size and direction of the inserted fragments were verified by PstI digestion. The sequencing indicated that HDGF2 cDNA fragment was correctly inserted into the vector.

Plasmids were transfected into CHO cells by lipofection with Lipofectin Kit (GIBco Life). After transfecting the cells for 48 hours and screening the cells with G418 for 2-3 weeks, the cells and cell supernatant were collected and the activity of the expressed protein was measured. G418 was removed and the transformants were subcultured continuously. The mixed clonal cells were limiting diluted and the subclones with higher protein activity were selected. The positive subclones were mass cultured by routine methods. 48 hours later, the cells and supernatant were collected. The cells were ultrasonicated. Using 50mM Tris-HCl (pH7.6) solution containing 0.05% Triton as an equilibrium solution and eluent, the active peak of the protein was collected with a pre-balanced Superdex G-75 column. Then, using 50mM Tris-HCl (pH8.0) solution containing 0-1 M NaCl as an eluent, the protein was gradiently washed on a DEAE-Sepharose column balanced with 50mM Tris-HCl (pH8.0) solution. The active peak of the protein was collected. The solution of the expressed protein was dialyzed with PBS (pH7.4), and finally lyophilized and preserved.

The molecular weight of the expressed protein was about 23 kDa as identified by 12% SDS-PAGE.

Moreover, the sequencing results of the 10 amino acids at the N- and C-terminal of the expressed protein indicated that they were identical to those in SEQ ID NO: 4.

Example 5

Antibody preparation

Antibodies were produced by immunizing animals with the recombinant proteins obtained in Examples 3 and 4. The method was as follows: the recombinant proteins were isolated by chromatography, and stored for use. Alternatively, the protein was isolated by SDS-PAGE electrophoresis, and obtained by cutting electrophoretic bands from gel. The protein was emulsified with Freund's complete adjuvant of the same volume. The emulsified protein was injected intraperitoneally into mice at a dosage of 50-100ug/0.2ml. 14 days later, the same antigen was emulsified with Freund's incomplete adjuvant and injected intraperitoneally into mice at a dosage of 50-100ug/0.2ml for booster immunization. Booster immunization was carried out every 14 days, for at least three times. The specific activity of the obtained antiserum was evaluated by its ability of precipitating the translation product of HDGF2 gene in vitro.

All the documents cited herein are incorporated into the invention as reference, as if each of them is individually incorporated. Further, it is appreciated that, in the above teaching of the invention, the skilled in the art can make certain changes or modifications to the invention, and these equivalents are still within the scope of the invention defined by the appended claims of the present application.

SEQUENCE LISTING

(1) General information:

(ii) Title of invention: NEW HUMAN HEPATOMA-DERIVED GROWTH FACTOR ENCODING SEQUENCE AND
POLYPEPTIDE ENCODED BY SUCH DNA SEQUENCE AND PRODUCING METHOD THEREOF

(iii) Number of Sequences: 8

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACCGCTCGTC CGCCCGGCTT GAG 23

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GATCCTAGAC ATGTATAAGT CTGCGC 26

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1024bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

1 ACCGCTCGTC CGCCCGGCTT GAGGCCGCG GGGAGCGCGC GCAATTCGTC GGCCCGCGGG
61 GGGGCGGCTT CCCGGCATCT TCGCGGCGAC CAAGGACTAC CAGGAAGGGG AGCGGCTGGG
121 ATGGCGCGTC CGCGGCCCGG CGAGTACAAA GCGGGCGACC TGGTCTTCGC CAAGATGAAG
181 GGCTACCCGC ACTGGCCGGC CCGGATTGAT GAACTCCAG AGGGCGCTGT GAAGCCTCCA
241 GCAAACAAGT ATCTATCTT CTTTTTGGC ACCCATGAAA CTGCATTCT AGGTCCCAAA
301 GACCTTTTTC CATATAAGGA GTACAAAGAC AAGTTTGGA AGTCAAACAA ACGGAAAGGA
361 TTTAACGAAG GATTGTGGGA AATAGAAAAT AACCCAGGAG TAAAGTTAC TGGCTACCAG
421 GCAATTCAGC AACAGAGCTC TTCAGAACT GAGGGAGAAG GTGGAAATAC TGCAGATGCA
481 AGCAGTGAGG AAGAAGGTGA TAGAGTAGAA GAAGATGGAA AAGGCAAAAG AAAGAATGAA
541 AAAGCAGGCT CAAAACGGAA AAAGTCATAT ACTTCAAAGA AATCCTCTAA ACAGTCCCGG
601 AAATCTCCAG GAGATGAAGA TGACAAAGAC TGCAAGAAG AGGAAAACAA AAGCAGCTCT
661 GAGGGTGGAG ATGCGGGCAA CGACACAAGA AACACAATT CAGACTTGCA GAAAACCACT
721 GAAGGGACCT AACTACCATA ATGAATGCTG CATATTAAGA GAAACCACAA GAAGGTTATA
781 TGTTTGGTTG TCTAATATTC TTGGATTGA TATGAACCA CACATAGTCC TTGTTGTCAT
841 TGACAGAACC CCAGTTTGTA TGTACATTAT TCATATTCCT CTCTGTTGTG TTTCGGGGGG
901 AAAAGACATT TTAGCCTTTT TTAAGTTA CTGATTAAAT TTCATGTTAT TTGGTTGCAT

961 GAAGTTGCCC TTAACCACTA AGGATTATCA AGATTTTTCG GCAGACTTAT ACATGTCTAG
1021 GATC

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 203 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: lineal

(ii) MOLECULE TYPE: polypeptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

1 Met Ala Arg Pro Arg Pro Arg Glu Tyr Lys Ala Gly Asp Leu Val
16 Phe Ala Lys Met Lys Gly Tyr Pro His Trp Pro Ala Arg Ile Asp
31 Glu Leu Pro Glu Gly Ala Val Lys Pro Pro Ala Asn Lys Tyr Pro
46 Ile Phe Phe Phe Gly Thr His Glu Thr Ala Phe Leu Gly Pro Lys
61 Asp Leu Phe Pro Tyr Lys Glu Tyr Lys Asp Lys Phe Gly Lys Ser
76 Asn Lys Arg Lys Gly Phe Asn Glu Gly Leu Trp Glu Ile Glu Asn
91 Asn Pro Gly Val Lys Phe Thr Gly Tyr Gln Ala Ile Gln Gln Gln
106 Ser Ser Ser Glu Thr Glu Gly Glu Gly Gly Asn Thr Ala Asp Ala
121 Ser Ser Glu Glu Glu Gly Asp Arg Val Glu Glu Asp Gly Lys Gly
136 Lys Arg Lys Asn Glu Lys Ala Gly Ser Lys Arg Lys Lys Ser Tyr
151 Thr Ser Lys Lys Ser Ser Lys Gln Ser Arg Lys Ser Pro Gly Asp
166 Glu Asp Asp Lys Asp Cys Lys Glu Glu Glu Asn Lys Ser Ser Ser
181 Glu Gly Gly Asp Ala Gly Asn Asp Thr Arg Asn Thr Thr Ser Asp
196 Leu Gln Lys Thr Ser Glu Gly Thr

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCACGGATCC ATGGCGCGTC CGCGGCCCC 29

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATCCGTCGAC TTAGGTCCT TCACTGGTT 29

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
CCCTAAGCTT ATGGCGCGTC CGCGGCCCC 29

5 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29bp

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
TTTCGGATCC TTAGGTCCT TCACTGGTT 29

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence encoding a polypeptide having human HDGF2 protein activity, wherein said nucleotide sequence shares at least 70% homology to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3, or said nucleotide sequence can hybridize to the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3 under moderate stringency.

2. The DNA molecule of Claim 1 wherein said nucleotide sequence encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 4.

3. The DNA molecule of Claim 1 wherein said nucleotide sequence comprises the nucleotide sequence of nucleotides 121-732 in SEQ ID NO: 3.

4. An isolated HDGF2 polypeptide comprising a polypeptide having the amino acid sequence of SEQ ID NO: 4, its active fragments, and its active derivatives.

5. The polypeptide of Claim 4 wherein said polypeptide is a polypeptide having the amino acid sequence of SEQ ID NO: 4.

6. A vector containing the DNA sequence of Claim 1.

7. A host cell transformed by the vector of Claim 6.

8. The host cell of claim 7 wherein it comprises E.coli.

9. The host cell of claim 7 wherein it comprises eukaryotic cell.

10. A method for producing a method for producing a polypeptide having the activity of HDGF2 protein, which comprises the steps of :

(a) forming an expression vector of HDGF2 protein comprising the nucleotide sequence encoding the polypeptide having the activity of HDGF2 protein, wherein said nucleotide sequence is operably linked with an expression regulatory sequences, and said nucleotide sequence shares at least 70% homology to the nucleotide sequence of positions 121-732 in SEQ ID NO: 3;

(b) introducing the vector of step (a) into a host cell, thereby forming a recombinant cell of HDGF2 protein;

(c) culturing the recombinant cell of step (b) under the conditions suitable for expression of HDGF2 polypeptides;

(d) isolating the polypeptides having the activity of HDGF2 protein.

11. The method of Claim 10 wherein said nucleotide sequence comprises nucleotides 121-732 of SEQ ID NO: 3.

12. An antibody specifically bound with the HDGF2 polypeptide of Claim 4.

13. A nucleotide molecule wherein it is the antisense sequence of the DNA molecule of Claim 1.

14. A probe wherein it comprises about 8-100 consecutive nucleotides of the DNA molecule of Claim

ABSTRACT

5 The invention provides a cDNA sequence of a new type II human hepatoma derived growth factor (HDGF2). The protein encoded by such sequence is a homology of type I HDGF. The present invention also relates to peptides encoded by the nucleotide sequences, to uses of these polynucleotides and polypeptides, and to methods for producing the said polynucleotides and polypeptides.

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Brian Mahanas
printed name

Brian Mahanas
signature

human	HDGF2	Nt	-	ACCGCTCGTCCGCCCCGGCTTGAGGCCCCGCGGGGAGCGCGCAATTCGTC	-50
mouse	HDGF	Nt	-	CGCAAAC-TTG	-10
human	HDGF2	Nt	-	GGCCCGCGGGGGGGCGGCCCTCCCGGCATCTTCGCGGCGACCAAGGACTAC	-100
mouse	HDGF	Nt	-	GGCTCGCGC-----TTCCCCGCT-CGGCGCGGAGCCCGG-GGCGCC	-49
human	HDGF2	Nt	-	CAGGAAGGGGAGCGGCTGGGATGGCGCG-TCCG--CGGCCCCGCGAGTAC	-147
mouse	HDGF	Nt	-	CG-----CGGCCCCGCCA---TGTCGCGATCCAACCGGCAGAAAGAGTAC	-91
human	HDGF2	Nt	-	AAAGCGGGCGACCTGGTCTTCGCCAAGATGAAGGGCTACCCGCACTGGCC	-197
mouse	HDGF	Nt	-	AAGTGCGGAGACCTGGTGTTCGGAAGATGAAAGGATACCCACACTGGCC	-141
human	HDGF2	Nt	-	GGCCCGGATTGATGAACTCCCAGAGGGCGCTGTGAAGCCTCCAGCAAACA	-247
mouse	HDGF	Nt	-	GGCCCGGATTGATGAGATGCCTGAGGCTGCAGTGAAGTCAACAGCCAACA	-191
human	HDGF2	Nt	-	AGTATCCTATCTTCTTTTTTGGCACCCATGAAACTGCATTTCTAGGTCCC	-297
mouse	HDGF	Nt	-	AATACCAAGTCTTTTTTTTTTGGGACCCATGAGACGGCATTCCTGGGCCCC	-241
human	HDGF2	Nt	-	AAAGACCTTTTTCCATATAAGGAGTACAAAGACAAGTTTGAAAAGTCAAA	-347
mouse	HDGF	Nt	-	AAAGACCTCTTCCCTTATGAGGAATCCAAGGAGAAGTTTGGCAAGCCCAA	-291
human	HDGF2	Nt	-	CAAACGGAAAGGATTTAACGAAGGATTGTGGGAAATAGAAAATAACCCAG	-397
mouse	HDGF	Nt	-	CAAGAGGAAAGGGTTCAGCGAGGGGCTGTGGGAGATCGAGAACAACCCCTA	-341
human	HDGF2	Nt	-	GAGTAAAGTTTACTGGCTACCAGGCAATTCAGCAACAGAGCTCTTC---A	-444
mouse	HDGF	Nt	-	CAGTCAAGGCCTCTGGCTACCAGTCTCTCCAGAAAAAGAGTTGTGCGGCA	-391
human	HDGF2	Nt	-	GAAAC-----TGAGGGAGAAGGTGGAAATAC---	-470
mouse	HDGF	Nt	-	GAGCCCGAGGTGGAGCCCGAAGCCCATGAGGGTGACGGTGATAAGAAGGG	-441
human	HDGF2	Nt	-	---TGCAGATGCAAGCAGTGAGGAAGAAGG-----TGATAGAGTA--	-507
mouse	HDGF	Nt	-	CAGTGCAGAGGGCAGCAGCGACGAAGAAGGGAAACTGGTGATCGATGAAC	-491

Fig. 1

human HDGF2 Nt - -----GAAGAAGATGGAAAAGGCAA-----AAGAA-AGA-----AT -537
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - CAGCCAAGGAGAAGAACGAAAAGGGCACGCTGAAGAGGAGAGCAGGGGAT -541

human HDGF2 Nt - G-----AAA-----AAGCAGGCTCAAAAC-----GGA -559
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - GTGTTGGAGGACTCCCCTAAACGTCCCAAGGAGTCAGGAGACCATGAGGA -591

human HDGF2 Nt - AAAAGTCAT---ATA---CTT-----CA----- -576
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - GGAGGACAAGGAGATAGCTGCCTTGAGGGTGAGAGGCACCTGCCTGTAG -641

human HDGF2 Nt - -----AAGAA-ATC-----CTCTAAAC-AGTC-----CCGGAATCT -606
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - AGGTGGAGAAGAACAGCACCCCCTCTGAGCCAGACTCTGGCCAGGGACCT -691

human HDGF2 Nt - CCAGGAGATGAAGATGACAAAGA-----CTGCAAAG-AAGAGG---A -644
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - CCTGCAGAGGAAGAAGAGGGAGAGGAAGAGGCTGCCAAGGAAGAGGCTGA -741

human HDGF2 Nt - A-----AA-----CAAA -651
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - AGCCCCAGGCGTCAGAGATCATGAGAGCCTGTAGCCACCAATGTTTCAAG -791

human HDGF2 Nt - AGCAGC-----TCTGAGGG-----TGGAGATGCG -675
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - AGGAGCCCCTGCCCCGTTCTGCTGCTGTCTGGGTGCTACTGGGGAAACT -841

human HDGF2 Nt - GGCAACGACA--CAA--GAA-----ACACAAC-- -699
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - GGCCATGGCCTGCAAACCTGGGAACCCCTTCCACCCCTATTTACCCTACTC -891

human HDGF2 Nt - --TCAG--ACT---TGCAGAAAACC-AGT---GAAG-----GGACCT -730
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - CCTCACTCACTCTCTCCTCTAAGCCCACTCCTGGAGAGTGTCTTGGCCCT -941

human HDGF2 Nt - AACTACCA-----TA-ATGAATGCTG---CATATTAAGAGA--AA -764
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - CACCTCCAGCTCCCTTCTATATACACCCTGTGCCCCAGGATGAGATGAG -991

human HDGF2 Nt - CCACAAGAAGGT-TATA---TGTTT-----GGTT-----GTCTAA -795
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - GCCTTTGTATCTCTTTACACTTGTTTCCCAGGGTTTCTGCTGGGGTCTAG -1041

human HDGF2 Nt - TAT-----TCTTG-----GA -805
 | | | | | | | | | | | | | | | | | | | | | |
 mouse HDGF Nt - GCTGCTGTTTCCACCTCTTGACACCTCTGCCCTGCTGCAGGCATTCTAGA -1091

Fig. 1 (cont.)

human HDGF2 Nt - --TTTG-----ATA-----TGAACCAACACATAG--- -827
 |||| ||| |||| ||| |||
 mouse HDGF Nt - CCTTTGGGGTGGATAGTGGGCAGGAGTGGAGGTGAAAGAATATAAAGGAG -1141

human HDGF2 Nt - -----TCCTTGTTGTCATTGAC-----AGAACC-----CCAG----- -854
 ||| ||| ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - TGTGGGTTTCATGGATGGCATCGTCTACCTGAGCTCCTGTCTCCAGCCCCC -1191

human HDGF2 Nt - -----TTTG----TATG--TACATT----- -868
 ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - ACACTTATTTTCCCATCTGCCTACATTCAAGAAACAGGACACTGTGGGAG -1241

human HDGF2 Nt - -----ATTCAT--ATTCCTCTCTGTTGTGTTTCGGG----- -897
 ||| ||| ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - AGAGGCTACCATCCATCCATAAATCCTTGTTGATTTTGGGAACACTTAT -1291

human HDGF2 Nt - -----GGGAA-AAGACATTTTAGC-----CTTT-- -919
 ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - CCCCTGACCCAGGGTTCAAGGAATTGTAGTTTAACATCTAGACTTTGG -1341

human HDGF2 Nt - --TTTAAAAGTT----- -929
 ||| ||||
 mouse HDGF Nt - AGTTTCCAAGTTTGGGCCTAGGACCTGGAGGGAGCTAAGAGCTGAAGAAT -1391

human HDGF2 Nt - --ACTGATTTAATTTCA-----TGT-TATTTGGTT-----GCATGAA-- -963
 ||||| ||| ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - CAACTGATTTGCATTGAGGAAATGTCTCTTTAGATCTCAGGGCAGAAATG -1441

human HDGF2 Nt - -----GTTGCCCTTAACCACT-----AAGGATTAT---C -989
 ||| ||| ||| ||| ||| ||| ||| |||
 mouse HDGF Nt - ATAACCTGGGGAGACCTGCTGCCTTCATCTACTTCCAATGCTTGAGGCC -1491

human HDGF2 Nt - A-----AGATTTTTCG-CGAGACTTATA-----CATGTCT- -1018
 | |||| ||| ||| |||| ||| ||| ||| |||
 mouse HDGF Nt - AGCCTGTAGTCAGATATTTACCCAGACATAAAGGAAAAGACCATTTTTT -1541

human HDGF2 Nt - --AGGATC -1024
 ||||
 mouse HDGF Nt - TTAGGAAATGTTTTTAATAAAAA -1563

Identity: 68.7%

Fig. 1 (cont.)

Identity: 53.7%
Similarity: 9.4%

Fig. 2

Attorney Docket No.

MERCHANT & GOULD P.C.

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NEW HUMAN HEPATOMA-DERIVED GROWTH FACTOR ENCODING SEQUENCE AND POLYPEPTIDE ENCODED BY SUCH DNA SEQUENCE
AND PRODUCING METHOD THEREOF

The specification of which

a. ☐ is attached hereto

b. ☒ was filed on _____ as application serial no. _____ and was amended on _____ (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/CN99/00139 filed 06.09.1999 and as amended on _____ (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.

b. ☒ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
China	98119758.2	22.09.1998	

ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

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I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant & Gould P.C. to the contrary.

Please direct all correspondence in this case to Merchant & Gould P.C. at the address indicated below:

Merchant & Gould P.C.
 P.O. Box 2903
 Minneapolis, MN 55402-0903



I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2	Full Name Of Inventor	Family Name	First Given Name	Second Given Name
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Signature of Inventor 201:			Date:	
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2	Full Name Of Inventor	Family Name	First Given Name	Second Given Name
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4	Post Office Address	Post Office Address	City	State & Zip Code/Country
Signature of Inventor 204:			Date:	
2	Full Name Of Inventor	Family Name	First Given Name	Second Given Name
0	Residence & Citizenship	City	State or Foreign Country	Country of Citizenship
5	Post Office Address	Post Office Address	City	State & Zip Code/Country
Signature of Inventor 205:			Date:	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim;

or

- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

- (1) Each inventor named in the application;
- (2) Each attorney or agent who prepares or prosecutes the application; and
- (3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.